[des-ALA^1,GLY^2]-HIS^{4,5} D-TRP^8 -SOMATOSTATIN

A glucagon-specific and long-acting somatostatin analog

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Received 5 June 1978

1. Introduction

The hypothalamic tetradecapeptide somatostatin [1], in addition to its growth hormone release inhibiting activity, lowers the basal levels of insulin and glucagon [2,3] and also suppresses the arginine-stimulated rise of these pancreatic hormones in man [4]. Unger et al. [5] have suggested that excessive release of glucagon either in basal or stimulated state may contribute to diabetic hyperglycemia.

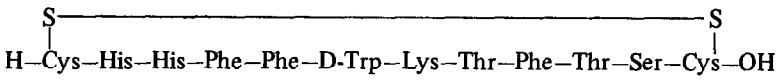
Somatostatin, soon after its discovery, was considered as a potential agent for the control of diabetes [6]. It became evident, however, that somatostatin would have limited use in clinical situations, due to a short, < 10 min, biological half-life [7] coupled with a diabetogenic activity resulting from the inhibition of the insulin secretion [8]. Analogs of somatostatin which would preferentially suppress glucagon without substantially affecting insulin and at the same time possess prolonged activity could be the answer to the above disadvantages of somatostatin. An analog, D-Trp^8–D-Cys^{14}-somatostatin, which shows relative specificity for the suppression of growth hormone and glucagon has been synthesized [9,10]. However, this analog does not show any long lasting activity for lowering growth hormone or glucagon [11].

We report here the synthesis and selective inhibitory activity towards growth hormone and glucagon of an analog of somatostatin [des-Ala^1,Gly^2]His^{4,5}-D-Trp^8-somatostatin, Wy-41,747, endowed with considerable prolongation of activity against these hormones.

2. Experimental

The dodecapeptide [des-Ala^1,Gly^2]-His^{4,5}-somatostatin, Wy-41,747, was synthesized by the solid phase peptide synthesis methodology [12]. The peptidoresin, Boc–Cys(SMBz1)–His(CBZ)–His(CBZ)–Phe–Phe–D-Trp–Lys(ClCBZ)–Phe–Thr(Bz1)–Ser(Bz1)–Cys(SMBz1)–Bz1 was treated with anhydrous HF in the presence of anisole and the disulfhydryl compound was oxidized by air to afford the cyclic disulfide.

The crude dodecapeptide disulfide was purified by gel filtration through Sephadex G-25 followed by partition chromatography through Sephadex G-25 with the biphasic system, *n*-butanol–water–acetic acid, 4:5:1, v/v/v. Amino acid analysis, Thr(2) 1.94, Ser(1) 0.89, Phe(3) 3, Lys(1) 1.01, His(2) 1.88,



[des-Ala^1,Gly^2]-His^{4,5}-D-Trp^8-somatostatin, Wy-41,747

Table 1
Effects of Wy-41,747 on suppression of growth hormone, insulin and glucagon at 15 min in rats

Peptide	Dose	Plasma hormone levels (M ± SEM)			Exp.
	(μg/kg)	Glucagon (pg/ml)	Insulin (μU/ml)	Growth hormone (ng/ml)	
None	–	33 ± 4	256 ± 18	142 ± 20	A
Wy-41,747	500	2 ± 2[a]	124 ± 28[a]	41 ± 6[a]	
None	–	69 ± 13	278 ± 32	163 ± 29	B
Wy-41,747	100	20 ± 8[a]	202 ± 49	20 ± 8[a]	
None	–	87 ± 10	699 ± 207	333 ± 38	C
Wy-41,747	20	35 ± 4[a]	569 ± 74	138 ± 34[a]	
Wy-41,747	5	70 ± 9	559 ± 131	188 ± 34[b]	

[a] $P < 0.01$
[b] $P < 0.05$ by analysis of variance

Trp(1) 0.82, Cys(2) 1.86. Thin-layer chromatography, cellulose precoated glass plates (Analtech) R_F (*n*-butanol–water–acetic acid, 4:1:1) 0.38, R_F (*n*-butanol–water–acetic acid–pyridine, 30:24:6:20) 0.69.

The hormone inhibiting profile at 15 min after subcutaneous injection at various doses of Wy-41,747, was measured by suppression of plasma growth hormone (GH) insulin (INS) and glucagon (GLUN) in arginine-stimulated rats anesthetized with nembutal as in [15]. The duration of action of Wy-41,747 in suppressing nembutal stimulated growth hormone release was determined as in [16].

3. Results

The favorable specificity for glucagon suppression by Wy-41,747 can be seen in table 1. The analog suppresses growth hormone at doses as low as 5 μg/kg and glucagon at a dose of 20 μg/kg, while insulin levels are lowered significantly by Wy-41,747 only at a dose of 500 μg/kg. A comparison between somatostatin and Wy-41,747 is presented in table 2, which shows that Wy-41,747 is more potent toward glucagon suppression and less potent toward growth hormone and insulin lowering.

Duration of activity of Wy-41,747 for growth hormone suppression is presented in table 3. A suspension of Wy-41,747 in saline will lower significantly GH levels in rats at a dose of 1 mg/kg, subcutaneously, for up to 2 h, while the same dose, 1 mg/kg, suspended in 80% polyethyleneglycol 400 solution will effectively lower GH for 4 h. Although Wy-41,747 did not lower glucagon for prolonged periods in the model described above, it did lower both glucagon and glucose in fasted streptozotocin diabetic dogs for 4 h at a dose of 0.5 mg/kg [17]. All of these results characterize Wy-41,747 as an ideal agent in the treatment of diabetes.

Table 2
Comparison of minimal effective doses (μg/kg) between somatostatin and Wy-41,747

	Somatostatin	Wy-41,747
Glucagon	50–100	5– 20
Insulin	100–200	100–500
GH	5– 10	5– 20

Acknowledgements

We thank Drs N. H. Grant and J. P. Yardley for helpful discussions and support, and Mr D. Clark for excellent technical assistance.

Table 3
Duration of plasma growth hormone suppression in rats

Peptide	Dose (μg/kg)	*N*	Time (h) after injection	Plasma growth hormone (ng/ml) M ± SEM
Saline	–	10	2	118 ± 20
Wy-41,747	1000	9	2	36 ± 6[a]
Saline	–	10	2	229 ± 37
Wy-41,747	500	9	2	48 ± 16[a]
Saline	–	9	4	115 ± 23
Wy-41,747	1000	10	4	62 ± 13
80% PEG-400	–	10	4	205 ± 24
Wy-41,747 in 80% PEG-400	1000	10	4	100 ± 12[a]
80% PEG-400	–	10	5	186 ± 20
Wy-41,747 in 80% PEG-400	1000	8	5	161 ± 21

[a] $p < 0.01$

N, number of animals per group

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